Respiratory Medicine (2009) **103**, 552–557

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Genetic association analysis of COPD candidate genes with bronchodilator responsiveness

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Received 14 July 2008; accepted 30 October 2008
Available online 25 December 2008

KEYWORDS
Bronchodilator responsiveness;
Chronic obstructive pulmonary disease;
Genetics;
Association analysis

Summary
Airflow limitation in COPD patients is not fully reversible. However, there may be large variability in bronchodilator responsiveness (BDR) among COPD patients, and familial aggregation of BDR suggests a genetic component. Therefore, we investigated the association between six candidate genes and BDR in subjects with severe COPD. A total of 389 subjects from the National Emphysema Treatment Trial (NETT) were analyzed. Bronchodilator responsiveness to albuterol was expressed in three ways: absolute change in FEV_1, change in FEV_1 as a percent of baseline FEV_1, and change in FEV_1 as a percent of predicted FEV_1. Genotyping was completed for 122 single nucleotide polymorphisms (SNPs) in six candidate genes (*EPHX1*, *SFTPB*, *TGFB1*, *SERPINE2*, *GSTP1*, *ADRB2*). Associations between BDR phenotypes and SNP genotypes were tested using linear regression, adjusting for age, sex, pack-years of smoking, and height. Genes associated with BDR phenotypes in the NETT subjects were assessed for replication in 127 pedigrees from the Boston Early-Onset COPD (EOCOPD) Study. Three SNPs in *EPHX1* ($p = 0.009$–0.04), three SNPs in *SERPINE2* ($p = 0.004$–0.05) and two SNPs in *ADRB2* (0.04–0.05) were significantly associated with BDR phenotypes in NETT subjects. One SNP in *EPHX1* (rs1009668, $p = 0.04$) was significantly replicated in EOCOPD subjects. SNPs in *SFTPB*, *TGFB1*, and *GSTP1* genes were not associated with BDR. In conclusion, a polymorphism of *EPHX1* was associated with bronchodilator responsiveness phenotypes in subjects with severe COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation that is not fully reversible; however, COPD patients are often treated with

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doi:10.1016/j.rmed.2008.10.025

bronchodilator medications.[1] There is large variability in bronchodilator responsiveness (BDR) among COPD patients, which has been related to various factors such as age,[2] smoking,[3] baseline lung function,[4] and eosinophil biomarkers in bronchoalveolar lavage fluid.[5] These findings suggest that differences in disease characteristics of COPD subjects may be associated with interindividual variation in pharmacological response to bronchodilator medications.

A previous paper found significant familial aggregation of BDR in the Boston Early-Onset COPD Study,[6] suggesting the effect of genetic factors. Recent studies revealed that polymorphisms in hemopoeitic cell kinase (*HCK*)[7] and β2-adrenergic receptor genes[8] may be associated with BDR in patients with COPD. However, the genetic determinants of BDR in COPD have not been definitively established.

Glutathione s-transferase pi 1 (*GSTP1*), microsomal epoxide hydrolase (*EPHX1*), transforming growth factor-β1 (*TGFB1*), serpin peptidase inhibitor clade E member 2 (*SERPINE2*), surfactant protein B (*SFTPB*) and β2-adrenergic receptor (*ADRB2*) are six candidate genes previously associated with COPD susceptibility in at least two studies.[9–13] *ADRB2* has been associated with BDR in subjects with asthma and COPD.[8,14] We hypothesized that genetic variants in these potential COPD susceptibility genes may explain some of the variability in bronchodilator responsiveness phenotypes. This may improve our understanding of COPD by identifying subsets of patients showing small or large bronchodilator responsiveness influenced by a particular COPD susceptibility gene. Therefore, we investigated the association between six candidate genes and BDR in two populations of subjects with severe COPD.

Methods

Subjects

The current analysis included 389 non-Hispanic white subjects in the National Emphysema Treatment Trial (NETT).[15] Subjects enrolled in NETT had severe airflow obstruction ($FEV_1 \leq 45\%$ predicted), hyperinflation, and bilateral emphysema on high-resolution chest CT. Spirometry was performed according to American Thoracic Society (ATS) standards before and after administration of 2 puffs (180 mcg) of inhaled albuterol.[16] CT analysis on NETT subjects has been described previously.[17]

Genes associated with BDR phenotypes in the NETT subjects were examined in participants in the Boston Early-Onset COPD Study. Enrollment of subjects and phenotyping in the Boston Early-Onset COPD Study have been described previously.[18] Spirometry was performed in accordance with ATS specifications before and after inhalation of 2 puffs (180 mcg) of albuterol using a spacer device. Extended pedigrees were ascertained through probands under 53 years old with COPD with $FEV_1 < 40\%$ predicted. The current analysis included 949 subjects in 127 pedigrees.

Both studies were approved by institutional review boards at participating centers. All subjects provided written informed consent.

Genotyping

Genotyping was done for 122 single nucleotide polymorphisms (SNPs) in six candidate genes (19 in *EPHX1*, 5 in *SFTPB*, 21 in *TGFB1*, 64 in *SERPINE2*, 7 in *GSTP1*, 6 in *ADRB2*) including upstream and downstream genomic regions. We used pairwise linkage disequilibrium (LD)-tagging in Tagger with[19] minimum minor allele frequency of 0.10 and r^2-threshold of 0.9, based on genotype data from Caucasian (CEU) trios in Phase II of the HapMap Project.[20] Additional SNPs were also genotyped, based on previously reported genetic association analyses of COPD-related phenotypes.[9–11] SNPs were genotyped using TaqMan (Applied Biosystems, Foster City, CA) or Sequenom (San Diego, CA) assays as previously reported.[21,22]

Statistical analysis

Response to albuterol was expressed in three ways: absolute change in FEV_1 [BDRABS = postbronchodilator FEV_1 − prebronchodilator FEV_1], change in FEV_1 as a percent of

Table 1 Baseline characteristics of 389 subjects in the National Emphysema Treatment Trial Genetics Ancillary Study and 949 subjects in the Boston Early-Onset COPD Study. Data are presented as means (±S.D.), unless otherwise noted.

Variable	NETT	Boston Early-Onset COPD Study		
		Probands	First degree relatives	Other pedigree members
Number	389	127	503	319
Male (%)	250 (64.3)	32 (25.2)	216 (42.9)	148 (46.4)
Age, years	67.4 ± 5.8	48.1 ± 4.7	42.0 ± 17.9	52.9 ± 18.2
Smoking, pack-years	66.4 ± 30.4	38.9 ± 21.9	17.3 ± 23.9	21.6 ± 26.5
Prebronchodilator FEV_1, % predicted	24.8 ± 6.6	19.2 ± 7.4	83.3 ± 21.9	84.9 ± 19.1
BDRABS[a]	0.09 ± 0.08	0.09 ± 0.07	0.11 ± 0.14	0.09 ± 0.13
BDRBASE[b]	13.3 ± 12.0	16.1 ± 13.5	4.9 ± 7.3	4.4 ± 6.6
BDRPRED[c]	3.2 ± 2.9	3.0 ± 2.6	3.3 ± 4.2	3.1 ± 3.9

[a] BDRABS = postbronchodilator FEV_1 − prebronchodilator FEV_1.

[b] $\text{BDRBASE} = \frac{(\text{postbronchodilator } FEV_1 - \text{prebronchodilator } FEV_1)}{\text{prebronchodilator } FEV_1} \times 100$

[c] $\text{BDRPRED} = \frac{(\text{postbronchodilator } FEV_1 - \text{prebronchodilator } FEV_1)}{\text{predicted prebronchodilator } FEV_1} \times 100$

baseline FEV_1 [BDRBASE = ((postbronchodilator FEV_1 − prebronchodilator FEV_1)/prebronchodilator FEV_1) × 100], and change in FEV_1 as a percent of predicted FEV_1 [BDRPRED = ((postbronchodilator FEV_1 − prebronchodilator FEV_1)/predicted FEV_1) × 100].

In NETT, associations between the three BDR phenotypes and SNP genotypes were tested using linear regression, adjusting for age, sex, and pack-years of smoking, assuming additive genetic models. Models for BDRABS were additionally adjusted for height. Analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC). Association between haplotypes and BDR phenotypes in NETT subjects was tested using haplo.stats.[23] In the Boston Early-Onset COPD Study, family-based association analysis was performed using PBAT software version 3.5[24] assuming additive genetic models, adjusting for age, sex, and pack-years of smoking. Models for BDRABS were additionally adjusted for height. A p-value ≤ 0.05 was used to define a statistically significant result.

Results

Baseline characteristics of subjects

The mean age of NETT subjects was 67.4 years, and mean baseline prebronchodilator FEV_1 was 24.8% predicted. Mean BDRABS was 0.09 L (range −0.17 to 0.45), 13.3% (range −16.5 to 67.9) for BDRBASE, and 3.2% (range −4.4 to 15.2) for BDRPRED (Table 1). Correlation between BDRABS and BDRBASE was 0.91, between BDRABS and BDRPRED was 0.94, and between BDRBASE and BDRPRED was 0.94 in NETT subjects. All subjects in NETT were required to abstain from smoking for 4 months prior to initial evaluation.[25] The mean age of probands in the Boston Early-Onset COPD Study was 48.1 years, and the mean baseline prebronchodilator FEV_1 was 19.2% predicted (Table 1). Sixteen out of 127 early-onset COPD probands (13%) were current smokers. Mean BDR phenotypes of probands in the Boston Early-Onset COPD Study were similar to those of NETT subjects (Table 1).

Association analysis in NETT subjects

As shown in Table 2, two SNPs in *EPHX1* (rs3753661 $p = 0.02$, rs3766934 $p = 0.02$) and two SNPs in *SERPINE2* (rs6712954 $p = 0.047$, rs7588220 $p = 0.01$) were significantly associated with BDRABS. Three SNPs in *EPHX1* (rs3753661 $p = 0.01$, rs3766934 $p = 0.009$, rs1009668 $p = 0.04$ (Fig. 1)) and two SNPs in *SERPINE2* (rs3795877 $p = 0.04$, rs7588220 $p = 0.01$) were associated with BDRBASE. One SNP in *EPHX1* (rs3766934 $p = 0.04$), 3 SNPs in *SERPINE2* (rs6712954 $p = 0.04$, rs3795877 $p = 0.03$, rs7588220 $p = 0.004$) and 2 SNPs in *ADRB2* (rs1042717 $p = 0.04$, rs1042718 $p = 0.045$) were associated with BDRPRED. The Arg16Gly (rs1041713) and Gln27Glu (rs1041714) polymorphisms in ABRD2 were not associated with BDR phenotypes. SNPs rs3753661 and rs3766934 of *EPHX1* were in complete LD ($r^2 = 1.0$). The three significant SNPs in *SERPINE2* were not in LD ($r^2 < 0.1$). SNPs in *SFTPB*, *TGFB1*, and *GSTP1* were not associated with BDR phenotypes.

Replication in Boston Early-Onset COPD Study Pedigrees

In order to assess replication of these associations, SNPs in *EPHX1*, *SERPINE2* and *ADRB2* were analyzed in the Boston Early-Onset COPD Study families. Among the significantly associated SNPs in the NETT subjects, one SNP downstream of *EPHX1* (rs1009668) was associated with BDRPRED ($p = 0.04$), and one SNP in intron 1 of *SERPINE2* (rs7588220) was associated with BDRBASE ($p = 0.003$) in the EOCOPD families. However, the direction of effect of *SERPINE2* SNP rs7588220 was not

Table 2 Genetic association results of bronchodilator responsiveness in subjects in the National Emphysema Treatment Trial. Associations for all three phenotypes are shown when any of the phenotypes had $p < 0.05$. Covariates included in the regression models included age, sex, and pack-years of smoking.

Gene	SNP	MAF	Role	BDRABS[a]		BDRBASE[b]		BDRPRED[c]	
				β (S.E.)	p	β (S.E.)	p	β (S.E.)	p
EPHX1	rs3753661	0.09	Intron	0.02 (0.01)	0.02	3.7 (1.5)	0.01	0.67 (0.35)	0.06
	rs3766934	0.09	Intron	0.03 (0.01)	0.02	4.0 (1.5)	0.009	0.74 (0.36)	0.04
	rs1009668	0.11	Downstream	−0.02 (0.01)	0.10	−3.0 (1.4)	0.04	−0.63 (0.35)	0.07
SERPINE2	rs6712954	0.06	Exon	−0.03 (0.01)	0.047	−3.8(2.3)	0.09	−1.11 (0.50)	0.04
	rs7588220	0.02	Intron	−0.06 (0.02)	0.01	−9.2 (3.5)	0.01	−2.44 (0.84)	0.004
	rs3795877	0.19	Intron	0.02 (0.01)	0.08	2.8 (1.3)	0.04	0.72 (0.32)	0.03
ADRB2	rs1042717	0.20	Exon	0.01 (0.01)	0.14	1.3 (1.1)	0.24	0.52 (0.26)	0.04
	rs1042718	0.17	Exon	0.01 (0.01)	0.16	1.3 (1.1)	0.27	0.54 (0.27)	0.045

MAF, minor allele frequency.

[a] BDRABS = postbronchodilator FEV_1 − prebronchodilator FEV_1.

[b] $\text{BDRBASE} = \frac{(\text{postbronchodilator } FEV_1 - \text{prebronchodilator } FEV_1)}{\text{prebronchodilator } FEV_1} \times 100$

[c] $\text{BDRPRED} = \frac{(\text{postbronchodilator } FEV_1 - \text{prebronchodilator } FEV_1)}{\text{predicted prebronchodilator } FEV_1} \times 100$

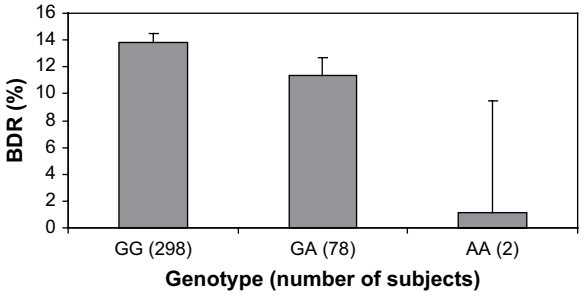

Figure 1 Bronchodilator response (BDRBASE) by rs1009668 coding SNP in NETT subjects. Mean values (+SEM) for change in FEV_1 as a percent of baseline FEV_1 are shown ($p = 0.04$). BDRBASE; change in FEV_1 as a percent of baseline FEV_1.

consistent in the two studies. SNP rs7588220 is relatively rare (minor allele frequency = 0.02) in EOCOPD families, so there were only a small number of informative families in PBAT analysis ($n = 3$). *EPHX1* SNP rs1009668 was associated with reduced BDR in the two study samples. SNPs in *ADRB2* were not associated with BDR phenotypes in EOCOPD families. However, rs1042713 (Arg16Gly) was associated with BDRPRED ($p = 0.04$) and BDRABS ($p = 0.04$) in subgroup with physician-diagnosed asthma history in the EOCOPD families.

Because SNP rs1009668 was the only replicated SNP with BDR across both populations, we examined associations with FEV_1 (% predicted) and chest CT emphysema. This SNP was not associated with FEV_1 level, but it was associated with increased percent emphysema on chest CT scans at a threshold of −950 HU ($\beta = 0.04$, $p = 0.009$) in NETT subjects.

Haplotype analysis

Haplotype analysis using haplo.stats in NETT subjects and using PBAT in EOCOPD families revealed associations similar to those found in the single SNP analysis in *EPHX1* (data not shown). In *ADRB2*, haplotypes of rs1042713 (Arg16Gly) and rs1042714 (Gln27Glu) were associated with BDRPRED in NETT (global $p = 0.046$), but this was not replicated in EOCOPD families.

Discussion

In this study, we examined SNPs in 6 COPD candidate genes including the *ADRB2* gene, which has been reported to be associated with bronchodilator responsiveness, and found associations between bronchodilator responsiveness in NETT subjects and SNPs in the *EPHX1*, *SERPINE2*, and *ADRB2* genes. The association between one SNP in *EPHX1* and bronchodilator responsiveness was replicated in extended pedigrees from the Boston Early-Onset COPD Study.

The product of *EPHX1* is microsomal epoxide hydrolase, which is important for the metabolism of cigarette smoke by-products. In previous studies, the fast allele of *EPHX1* (His139Arg, rs2234922) was found to be protective against COPD in a case—control study comparing NETT cases to control smokers,[9] protective against upper lobe predominant emphysema in NETT,[17] and associated with improved response to lung volume reduction surgery (LVRS), as measured by BODE score.[22] The slow allele of *EPHX1* (Tyr113His, rs1051740) has been associated with emphysema susceptibility[26] and reduced lung function.[27] Haplotype analysis of His139Arg and Tyr113His revealed associations with rapid lung function decline in the Lung Health Study.[28] In NETT, other SNPs in *EPHX1* have been associated with exercise capacity, DLCO and response to LVRS.[21,22]

The replicated SNP, rs1009668, was chosen because it is downstream of *EPHX1*, but is actually located in exon 1 of an adjacent gene, *KIAA0792*. The function of this gene product is not known, but the gene has been shown to be expressed in adenocarcinoma of the lung.[29] This SNP results in replacement of valine with methionine at amino acid position 622. In COPD patients, lower BDR may be related to more severe emphysema.[4] This SNP (rs1009668), which was associated with lower BDR, was also associated with increased emphysema and may have a role in COPD pathogenesis. However, it is not clear whether this SNP association is due to linkage disequilibrium with a functional variant in *EPHX1* or in *KIAA0792*.

An SNP in intron 1 of *SERPINE2* (rs7588220) was associated with BDR in both NETT subjects and Boston Early-Onset COPD Study families, although the directionality of association was not consistent. This gene was associated with COPD susceptibility in several COPD populations.[11,30] However, the role of this gene in the pathophysiology of COPD has yet to be identified. It is possible that *SERPINE2* variants could define a subset of COPD patients with differential bronchodilator responsiveness.

Two previous studies have investigated the association between BDR and genetic polymorphisms in COPD. A recent publication reported an association of BDR and polymorphisms of the β2-adrenergic receptor gene (*ADRB2*) in 246 Japanese patients with COPD.[8] Arg16 genotypes and Arg16—Gln27 haplotypes were associated with decreased BDR. This gene has been studied as a susceptibility gene for asthma[31] and COPD,[12,13] and also for a direct role in drug response.[32] Studies in asthma suggest the Arg16 allele is associated with greater acute bronchodilator responsiveness but decreased long-term response to regular use of short-acting β2 agonists.[14]

In our study, there was association only with haplotypes of the codon 16 and 27 polymorphisms in NETT subjects, but this was not replicated in EOCOPD families. In the EOCOPD families, the codon 16 polymorphism was associated with BDR only in the subgroup of subjects with a history of physician-diagnosed asthma. This suggests that *ADRB2* is not a major determinant of BDR in COPD, but may be relevant in patients with an asthmatic phenotype.

In another study,[7] a polymorphism in the hemopoietic cell kinase (*HCK*) gene was associated with differential expression of Hck protein and myeloperoxidase release from polymorphonuclear leukocytes in 60 COPD patients. This polymorphism was associated with BDR in 487 subjects from the Lung Health Study, suggesting that this gene contributes to COPD pathogenesis and modifies BDR. Because this gene was not associated with COPD susceptibility, we did not include it in our candidate gene panel.

The present study has several limitations. First, because there is not a single optimal measurement for bronchodilator responsiveness, we analyzed bronchodilator phenotypes expressed in three ways. All three measurements have their advantages and limitations, so we investigated all three phenotypes and used a replication strategy in this study to limit the possibility of false positive results. Second, the replicated SNP association in *EPHX1* was for a different, but highly correlated, BDR phenotype in our two study populations. Third, intra-individual variability in response to bronchodilator[33] may reduce power to detect genetic associations, yet we were still able to identify SNP-level replication for association between *EPHX1* and BDR. Fourth, the LD-tagging approach yielded SNPs that may not be the specific functional variants. The associated SNPs were intronic or in an exon of a gene with unknown function. The actual causal variant or variants remain to be discovered. Fifth, multiple statistical comparisons are a potential concern in any complex disease genetics study. Though the optimal approach to adjust for multiple testing is not clear, we used replication of the results in another study sample to guard against false positive results.

The NETT subjects and the Boston Early-Onset COPD probands all have severe airflow obstruction. Thus, the applicability of our findings to mild-to-moderate COPD patients may be limited. However, family members in the Boston Early-Onset COPD Study had a broad range of lung function values. Early-Onset COPD subjects likely represent a unique subgroup of COPD patients. This could contribute to lack of replication of our associations found in NETT.

In conclusion, polymorphisms of *EPHX1*, *SERPINE2*, and *ADRB2* were associated with bronchodilator responsiveness phenotypes in one population of subjects with severe COPD. BDR in COPD patients may depend upon different disease subtypes, differences in drug metabolism, or other pharmacogenetic effects. This study revealed that *EPHX1* — which has been previously associated with COPD susceptibility, lung function and CT phenotypes — demonstrated replication in a second population. BDR may be partly explained by genetic factors. Future directions will include replication in additional populations, identification of the functional variant or variants, and determination whether there are subtypes of COPD with differential bronchodilator responsiveness.

Conflict of interest statement

WK, CH, DD and JR have no conflicts of interest to disclose. ES received an honorarium from Bayer for a symposium at the ERS Meeting in 2005; an honorarium for a talk on COPD genetics in 2006, and grant support and consulting fees from GlaxoSmithKline for two studies of COPD; and he received an honorarium from Astra—Zeneca for a talk at the Lund Symposium in 2007 as well as consulting fees.

Acknowledgement

Funding: This study was supported by NIH grants HL075478, HL71393, HL080242, HL072918, HL083069, and the Alpha-1 Foundation. The National Emphysema Treatment Trial (NETT) was supported by contracts with the National Heart, Lung, and Blood Institute (N01HR76101, N01HR76102, N01HR76103, N01HR76104, N01HR76105, N01HR76106, N01HR76107, N01HR76108, N01HR76109, N01HR76110, N01HR76111, N01HR76112, N01HR76113, N01HR76114, N01HR76115, N01HR76116, N01HR76118, and N01HR76119), the Centers for Medicare and Medicaid Services, and the Agency for Healthcare Research and Quality.

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